SINGLE CELL ANALYSIS USING SECONDARY ION MASS SPECTROMETRY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Pursuant to 35 U.S.C. § 119(e), this application claims the benefit of U.S. Provisional Patent Application Nos. 62/138,322, filed Mar. 25, 2015, which application is incorporated herein by reference in its entirety.

GOVERNMENT RIGHTS

[0002] This invention was made with Government support under contract W81XWH-12-1-0591 awarded by the Department of Defense and under contracts GM104148 and HHSN268201000034C awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND

[0003] Cell-based methods for the detection of biomarkers in biological samples, such as blood samples, are important for many applications, including medical diagnostics, disease monitoring and prognosis, and drug discovery. With the current growth and future potential of personalized medicine, there is an increasing demand for rapid, high-throughput and sensitive methods to detect a large number of disease- and individual-specific biomarkers in order to provide personalized diagnoses and therapies to patients. However, most cell-based methods are limited in their multiplexing capabilities, speed, resolution and/or sensitivity.

[0004] Flow cytometry, such as fluorescence-activated cell sorting (FACS), is a well-known method for classifying cells and detecting biomarkers based on optical properties of labeled cells. However, conventional flow cytometry is limited in the number of labels that can be used simultaneously because of the spectral overlap between different labels. Additionally, these conventional light-based cytometric methods lack the resolution to provide subcellular distribution of biomarkers—a property that could be related to their activation state or function.

[0005] As an alternative to detecting optical signals from a sample, methods to detect molecular mass signatures of a sample using mass spectrometry are known. For example, inductively coupled plasma mass spectrometry (ICPMS) has been used to perform single-cell analysis of a sample by spraying single-cell droplets into an inductively coupled argon plasma to vaporize each cell and ionize the atomic constituents (Bendall et al., Science 2011 332:687). However, ICPMS-based methods are limited in speed, sensitivity, recovery of samples, and inherently does not reveal information related to subcellular localization of labeled targets.

SUMMARY

[0006] Described herein is a way to analyze a population of cells which, in certain embodiments, involves making an array of mass-tag labeled cells on a substrate, and analyzing the cells on a cell-by-cell basis using secondary ion mass spectrometry (SIMS). Depending on how the method is implemented, the present method may be done with a high level of multiplexing and with single-molecule sensitivity. In addition, the present method may be done in a high throughput way, and the subcellular location of the labels can be determined. Further, the analyzed cells can be recovered for further analysis. None of these potential advantages is achievable by conventional single cell analysis methods.

[0007] In certain embodiments, the method may involve: i) obtaining an array of cells on a substrate, wherein the cells are labeled with one or more mass tags and are separated from one another, ii) measuring, using secondary ion mass spectrometry (SIMS), the abundance of the one or more mass tags at a plurality of locations occupied by the cells, thereby generating, for each individual cell measured, a set of data, and iii) outputting the set of data for each of the cells analyzed. The array of cells may be an addressable array, or a random array. In embodiments of the method where the array of cells is a random array, the locations on the substrate occupied by the cells are determined by imaging the substrate prior to the measuring step. In some embodiments, the imaging is by optical imaging, electron imaging or low resolution SIMS.

[0008] In any embodiment, the measuring step may include applying a SIMS ion beam with a diameter equal to or greater than half the average diameter of individual cells to measure the abundance of the one or more mass tags on a whole cell basis. In such embodiments, the ion beam may have a diameter in the range of 1 μm to 50 μm.

[0009] In any embodiment, the measuring step may include applying a plurality of pulses of a SIMS ion beam at different sites of an individual cell of the array to obtain measurements of the abundance of the one or more mass tags at the different sites. In such embodiments, the ion beam may have a diameter in the range of 10 nm to 1500 nm. In certain embodiments, the method may include measuring the abundance of the one or more mass tags at a plurality of depths as the SIMS ion beam etches through the individual cell.

[0010] In any embodiment, the array of cells may be obtained by labeling cells with one or more mass tags, and attaching cells to a substrate, wherein the labeling is done either prior to or after the cells are attached to the substrate. The labeling may be done by administering the mass tag to an animal subject and obtaining labeled cells from the subject.

[0011] In any embodiment, the method may include labeling cells with a first mass tag and a second mass tag, wherein the first mass tag localizes to a known subcellular structure of the cell, measuring the abundance of the first and second mass tags at different sites of an individual cell of the array, and determining the subcellular localization of the second mass tag based on the measured abundance of the first and second mass tags.

[0012] In any embodiment, the method may further include identifying one or more cells of interest based on the obtained set of data, and recovering the identified cells for further analysis.

[0013] In any embodiment, the method may include remeasuring the abundance of the one or more mass tags at a plurality of locations occupied by the identified cells of interest, using SIMS, thereby generating, for each individual cell remeasured, a second set of data, and outputting the second set of data for each of the identified cells of interest.

[0014] Also disclosed herein is a method of analyzing a test population of cells. In these embodiments, the method may involve obtaining an array of cells on a substrate, wherein the cells are labeled with one or more mass tags and are separated from one another; measuring, using SIMS, the abundance of the one or more mass tags at a plurality of